AD_____

Award Number: W81XWH-04-1-0242

TITLE: Therapy Selection by Proteomic Profiling

PRINCIPAL INVESTIGATOR: Simon W. Hayward, Ph.D.

CONTRACTING ORGANIZATION: Vanderbilt University Medical Center
Nashville, TN 37232-2765

REPORT DATE: February 2005

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

# REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 074-0188



| 1. AGENCY USE ONLY | 2. REPORT DATE | 3. REPORT TYPE AND DATES COVERED |
|---|---|---|
| | February 2005 | Annual (26 Jan 2004 - 25 Jan 2005) |

**4. TITLE AND SUBTITLE**
Therapy Selection by Proteomic Profiling

**5. FUNDING NUMBERS**
W81XWH-04-1-0242

**6. AUTHOR(S)**

Simon W. Hayward, Ph.D.

**7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)**
Vanderbilt University Medical Center
Nashville, TN 37232-2765

E-Mail: simon.hayward@vanderbilt.edu

**8. PERFORMING ORGANIZATION REPORT NUMBER**

**9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)**
U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

**10. SPONSORING / MONITORING AGENCY REPORT NUMBER**

**11. SUPPLEMENTARY NOTES**

**12a. DISTRIBUTION / AVAILABILITY STATEMENT**
Approved for Public Release; Distribution Unlimited

**12b. DISTRIBUTION CODE**

**13. ABSTRACT (Maximum 200 Words)**

The **long term goal** of this work is to develop a new prognostic tool with which to determine the response of a patient to a given therapy, with the view of providing the most appropriate treatments tailored to individual patients. The **central hypothesis** of this proposal is that a subset of the proteins expressed in a prostate tumor can be used to predict response to specific therapeutic regimens. The **purpose** of this work is to generate predictive methods which will allow patients to be selected for specific treatment protocols. In this year, per our proposed schedule, we have focused on acquisition of tissue samples and their grafting and treatment in SCID mouse hosts. In line with DAMD 17-03-1-0047, a modification of the control arm of the study has been made to reflect changes in clinical practice occurring around the inception of the study which are likely to persist for the foreseeable future. Other minor technical improvements have also been made relating to the microarray plans and the method for detecting apoptotic cells. The project is proceeding on its predicted timeline as outlined in the accepted statement of work.

**14. SUBJECT TERMS**
Proteomic profiling, Taxotere, Prediction of response, biostatics

**15. NUMBER OF PAGES**
8

**16. PRICE CODE**

| 17. SECURITY CLASSIFICATION OF REPORT | 18. SECURITY CLASSIFICATION OF THIS PAGE | 19. SECURITY CLASSIFICATION OF ABSTRACT | 20. LIMITATION OF ABSTRACT |
|---|---|---|---|
| Unclassified | Unclassified | Unclassified | Unlimited |

Table of Contents

**Annual Report**
PCRP Idea Development Award
W81XWH-04-1-0242
Therapy Selection by Proteomic Profiling
**P.I. Simon W. Hayward, PhD**

## Introduction

The **long-term goal** of this work is to develop a new prognostic tool with which to determine the response of a patient to a given therapy, with the view of providing the most appropriate treatments tailored to individual patients. The **central hypothesis** of this proposal is that a subset of the genes expressed in a prostate tumor can be used to predict response to specific therapeutic regimens. The **purpose** of this work is to generate predictive methods that will allow patients to be selected for specific treatment protocols. The **rationale** is to utilize a novel method of human prostate cancer tissue xenografting in combination with state of the art proteomic, biostatistical and bioanalytical analysis to generate new prognostic tools. This project is an essential "proof of principle" step in the sense that if this methodology is successful with Taxotere it should be applicable to any new therapeutic approach that exists or which will be developed in the future. This work will allow us to design new predictive proteomic assays to determine whether specific patients will respond to Taxotere. This project is linked to, and shares tissue resources with a related study DAMD 17-03-1-0047 which has similar aims in terms of gene expression. The funding of these two projects running in parallel with the same patient samples allows the possibility of mixed genomic/proteomic-based tool development.

**Statement of Work**
PCRP Idea Development Award
W81XWH-04-1-0242
Therapy Selection by Proteomic Profiling
**P.I. Simon W. Hayward, PhD**

Task 1
Generate MALDI-MS profiles for prostate cancer samples from 260 patient tumor samples

a) As cases present, collect 260 histopathologically-confirmed prostate cancer tissue cores. Snap freeze core fragments (months Underway-24)

b) Cut adjacent frozen sections onto slides and onto MALDI-MS target plates; identify areas of tumor by H&E staining (months 1-25)

c) Run MALDI-MS analysis of 260 samples, store data sets electronically (months 1-25)

Task 2.
a) Graft tissues from the cores used in task 1 to pairs of SCID mice (months 1-24)

c) Treat one of each pair of mice with Taxotere for 30 days (months 1-25)

d) Sacrifice mice and harvest tissues. (months 2-26)

e) Perform TUNEL and histopathologic analysis of both control and Taxotere-treated samples. Calculate apoptotic indices (months 3-28).

This task requires the use of 260 male SCID mice (note that the first 150 of these samples will be generated as a part of DAMD 17-03-1-0047 and thus only 120 mice are requested upon this application, 110 for defined experiments and an extra 10 mice to act as backups in case of perisurgical mortality).

Task 3
Biostatistical analysis (to be performed by the VICC Biostatistics Core, funded through internal sources).

a) Identification of protein expression profiles which predict histopathologic response to Taxotere. Biostatistical analysis to determine a protein expression profile in tissue cores which predict histopathologic response to Taxotere in a xenograft model (months 26-32).

b) Identification of proteins regulated by Taxotere in responsive and non-responsive tissues. Biostatistical and bioinformatic analysis will be used to identify protein peaks regulated by Taxotere in responsive and non-responsive tissue samples (months 28-34).

Task 4
Prediction of response of patients in a clinical trial setting (months 35-36)

Based upon MALDI-MS analysis of archived snap frozen tissue the ability of the protein expression patterns identified in task 3 to predict response in a clinical trial will be tested. Run MALDI-MS analysis on samples; predict response based upon data acquired in earlier tasks. Test results by breaking patient code and correlating actual and predicted responses.

Summary of the Project
**Work Ongoing and Completed**
PCRP Idea Development Award
W81XWH-04-1-0242
Therapy Selection by Proteomic Profiling
**P.I. Simon W. Hayward, PhD**

The main tasks for the initial year of this proposal have been, 1) to collect and graft tissues into SCID mice, and 2) treat the mice with Taxotere and subsequently (six days post-treatment) to harvest the tissues for analysis. This procedure has been followed for a total of 96 patient samples. Control samples have been snap frozen. Formalin fixed sections of treated tissues have been prepared for analysis of apoptotic indices. In order to provide good internal comparison and to give numbers which can realistically be examined by biostatisticians, analysis will proceed in batches as samples accumulate.

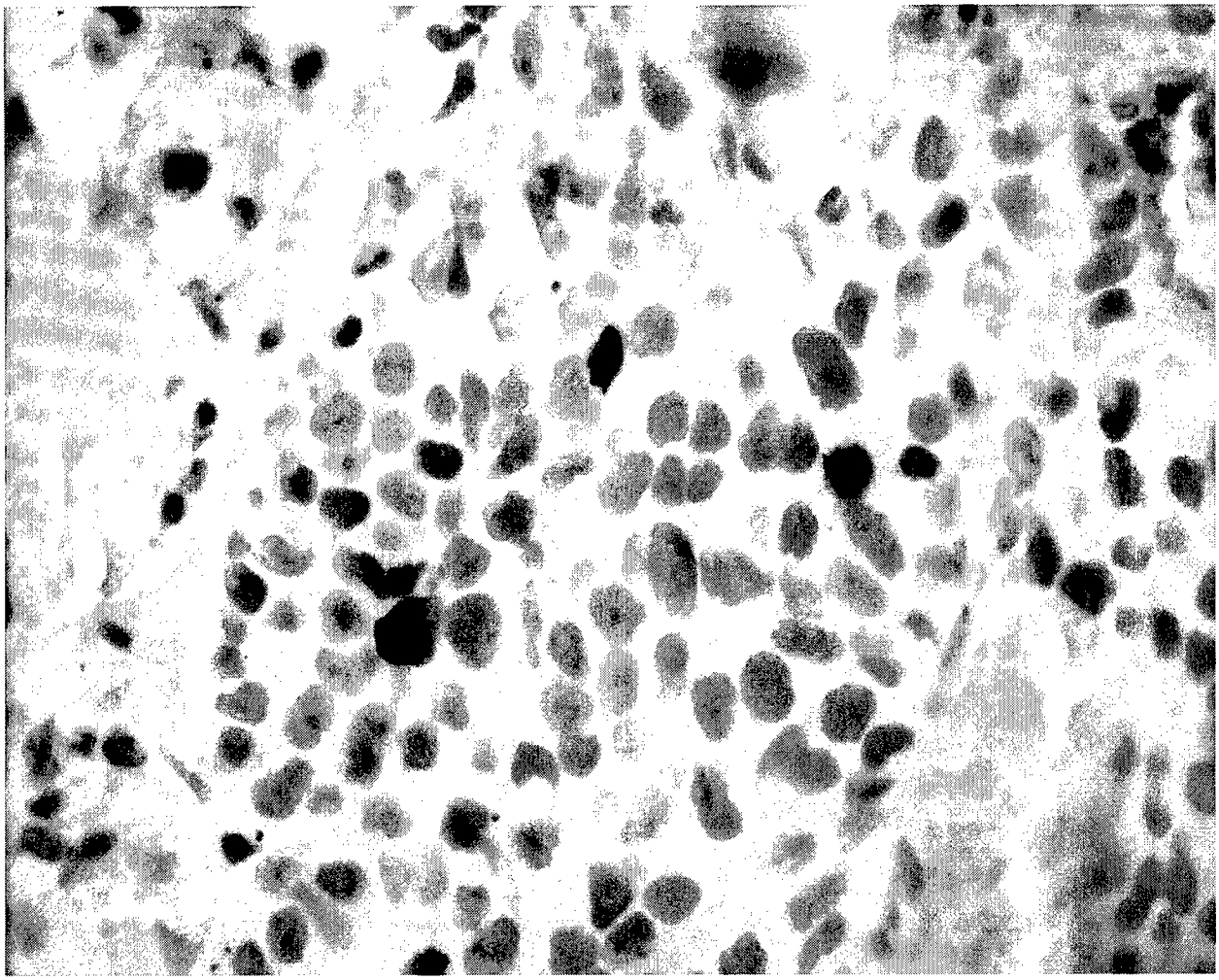
**Technical modifications:**

Clinical Practice Changes

As noted in the first annual report of a related study DAMD 17-03-1-0047 one major technical issue has caused us to modify the originally proposed procedure. Around the start of this study there was a technical modification to the process of prostatectomy at VUMC with the introduction of robotic techniques. This reflects changes in surgical practice occurring at many major hospitals across the USA. A consequence of this change is that the tissues spend significantly more time inside the body of the patient between their surgical removal from the blood supply and final delivery to Pathology. This can compromise the quality of the tissues by the time they become available to investigators, and certainly will change the profile of genes and proteins expressed by the cells. Therefore we have modified the protocol to use tissues grafted to untreated mice as the source for test profiles for this study. The act of grafting into a SCID mouse host provides an opportunity for the tissues to recover and regain their original profile of markers. This modification to task one does not change the overall goals of the project.

Analysis of Apoptotic Index

In the grant proposal we suggested that TUNEL assays would be used to determine apoptotic index following Taxotere treatment. TUNEL has well-recognized technical difficulties and while we have a significant level of experience with the technique we have decided to use a newly available immunohistochemical approach based upon antibodies against activated caspase 3, which is localized to the nucleus specifically in cells undergoing apoptosis (see figure below). This method is both technically simpler and more reliable than TUNEL and was accepted in its annual report as a modification to the related study DAMD 17-03-1-0047. We therefore propose to use the same modification in this project.



Activated caspase 3 staining in an LNCaP tumor. Note blood lakes characteristic of an LNCaP tumor. Intense brown stained nuclei indicate active apoptosis.

**Personnel Changes**

Dr. Scott Shappell recently left Vanderbilt to enter private clinical practice. Human prostate cancer tissues are now obtained on a recharge basis through the VICC Tissue Acquisition Core under the directorship of Dr. Kay Washington. Dr. Washington ensures that tissue cores are collected by the same protocol used by Dr. Shappell and coordinates delivery of samples from the OR through Pathology to the laboratory. Either Dr. Washington or Dr. Marcia Wills (Department of Pathology) provides a timely, core-by-core, histopathologic analysis based upon frozen sections of fractions of each core as previously performed by Dr. Shappell.

**Key Research Accomplishments**

- Grafting of tissue samples to SCID mice in control and treatment study arms.
- Treating mice with Taxotere.
- Harvesting and subsequent processing of tissues.

**Reportable Outcomes.**

None

**Conclusions.**

This work is proceeding on the predicted timeline. A number of changes to the specific details of the original statement of work are noted. These reflect changes in clinical practice that is beyond the control of the investigators and technical methodological improvements that enhance the overall quality of the proposal. None of these changes alters the overall aims and long-term goal of the proposed work.